

Development of a lateral flow immunoassay to detect crustaceans in processed food

Clara Esteban ^a, Alba Civera ^a, Alba Gutiérrez ^a, Patricia Galan-Malo ^b, Luis Mata ^b, Lourdes Sánchez ^a, María D. Pérez ^a

^a Departamento de Producción Animal y Ciencia de los Alimentos. Facultad de Veterinaria. Instituto Agroalimentario de Aragón (IA2)

(Universidad de Zaragoza-CITA), Miguel Servet, 177, 50013 Zaragoza, Spain

^b ZEULAB S.L., Polígono PLAZA, Bari, 25 Duplicado, 50197 Zaragoza, Spain



Facultad de Veterinaria
Universidad Zaragoza



Instituto Universitario de Investigación Mixto
Agroalimentario de Aragón
Universidad Zaragoza



3rd FOOD CHEMISTRY Conference
Shaping a Healthy and Sustainable Food Chain Through Knowledge
10-12 October 2023 • Dresden, Germany

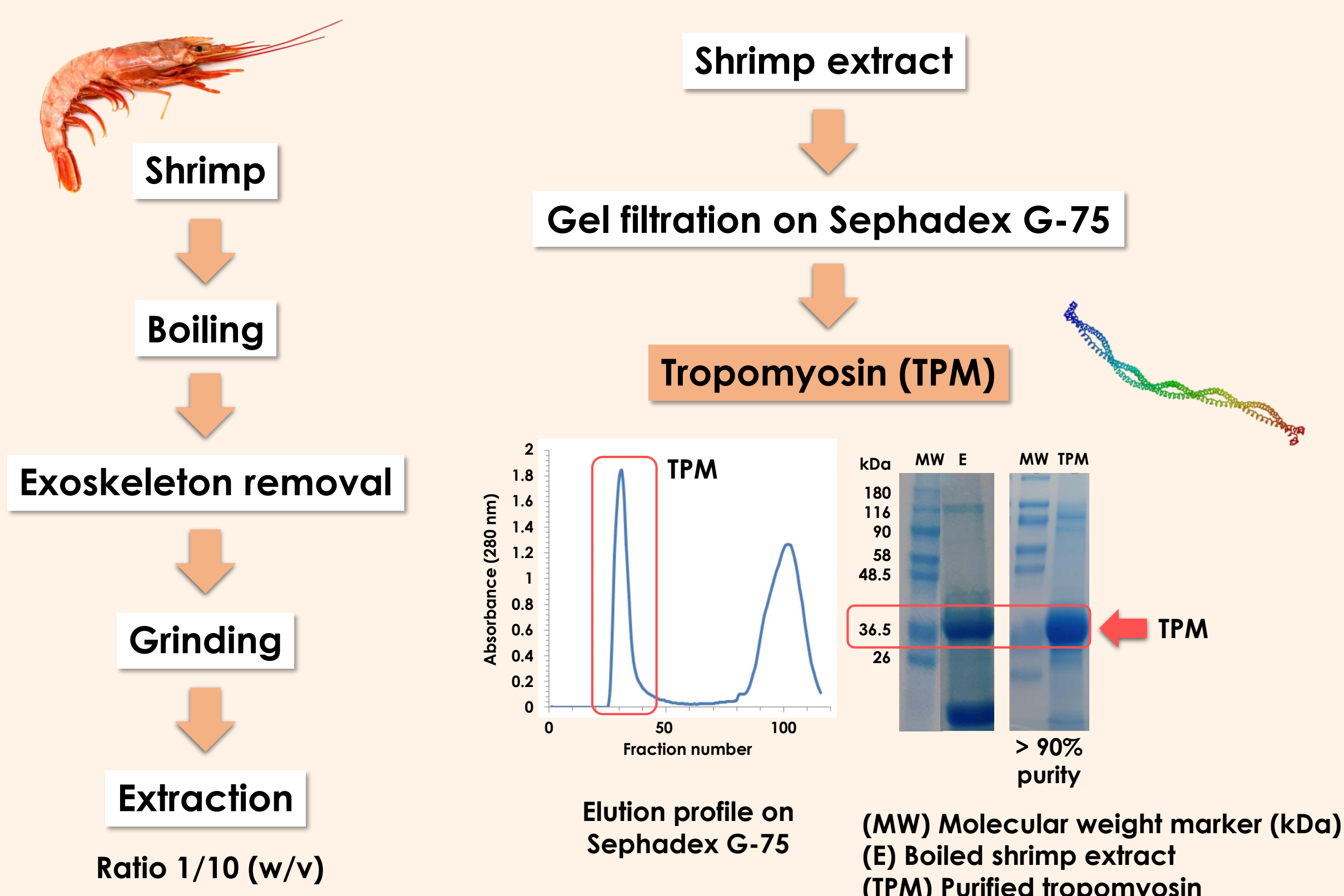


INTRODUCTION

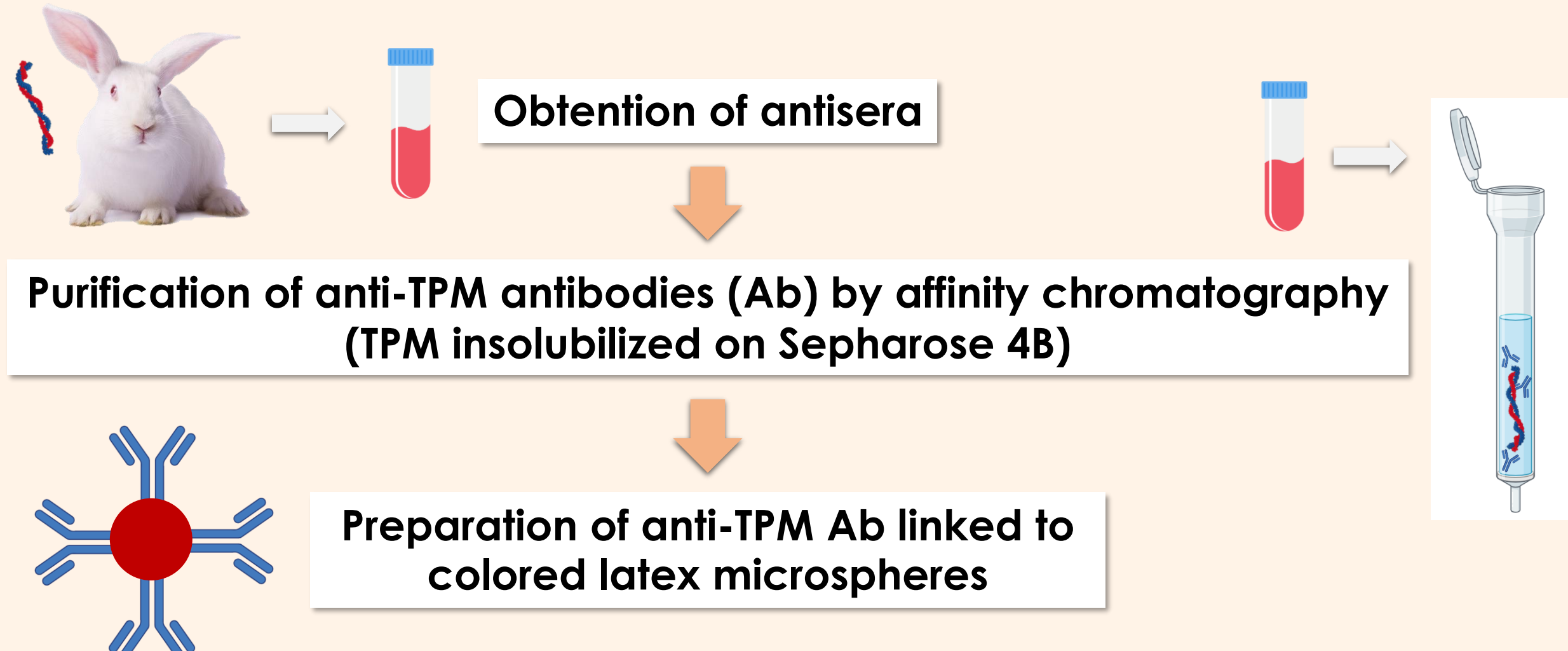
Crustaceans are among the shellfish most commonly consumed and one of the main triggers of food allergy, being the third most important cause of food-induced anaphylaxis. According to current labeling regulations, it is mandatory to include crustaceans if they are used as food ingredients. However, they can also be present as hidden allergens due to cross-contamination during processing. For this reason, specific and sensitive analytical techniques are required to control the unintended presence of crustaceans in food to protect allergic consumers. The aim of this study was the development and validation of a lateral flow immunoassay (LFIA) to detect the presence of traces of crustaceans in processed food.

EXPERIMENTAL

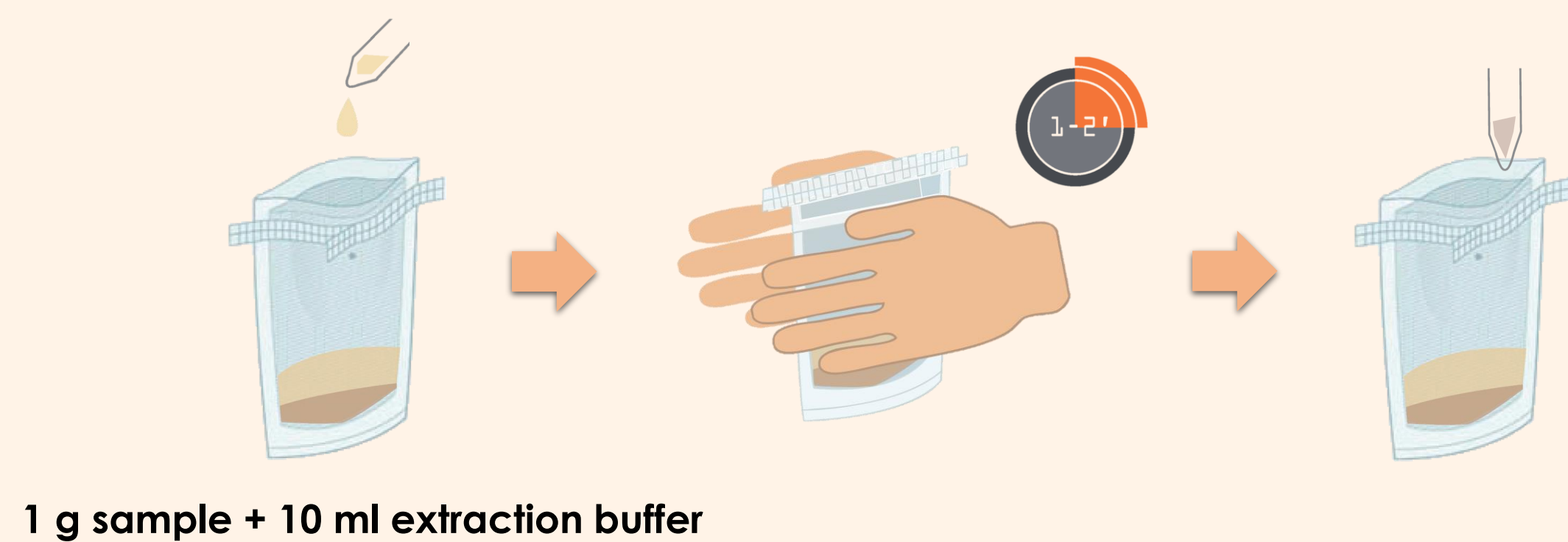
TROPOMYOSIN PURIFICATION



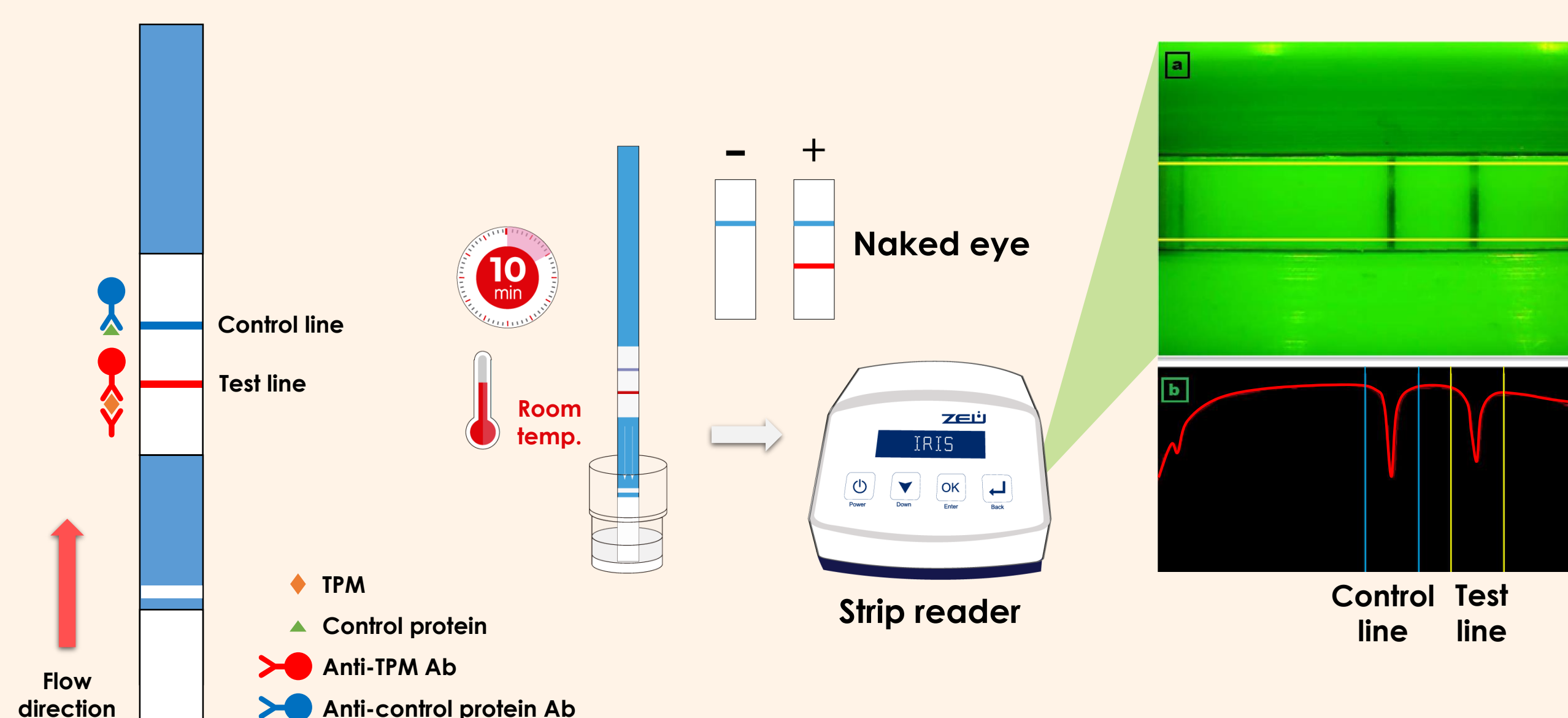
ANTIBODY PURIFICATION AND LABELING



EASY SAMPLE EXTRACTION



SANDWICH LFIA



RESULTS

CROSS-REACTIVITY OF BASIC INGREDIENTS

Ingredient	TL	Ingredient	TL	Ingredient	TL	Ingredient	TL	Ingredient	TL
Turmeric	0.4	Lenil	0.5	Brazil nut	0.6	Tuna	0.8	Squid	28.6
Curry	0.4	Chickpea	0.1	Pecan nut	0.8	Anchovy	0.9	Scallop	14.3
Cinnamon	1.6	Lupin	0.7	Apple	0.6	Surimi	0.6	Octopus	36.9
Nutmeg	1.7	Peanut	1.2	Kiwi	0.1	Canned mussels	1.6	Clam	134.4
White pepper	0.7	Honey fried peanut	0.4	Carrot	0.3	Wheat flour	0.0	Cricket	19.6
Black pepper	0.7	Quinoa	1.9	Pork	0.6	Shredded coconut	0.3	Mealworm	134.9
Thyme	0.3	Rye	1.1	Chicken	0.4	Cocoa powder	1.0		
Rosemary	0.3	Rice flour	0.3	Beef	0.2	Whole milk	0.8		
Oregano	0.6	Cornstarch	0.5	Whiting	0.4	Orange juice	1.0		
Green anise	1.3	Cashew nut	1.0	Cod	0.5	Red wine	0.8		
Chopped garlic	0.5	Hazelnut	0.5	Salmon	1.1	Sugar	1.0		
Asturian beans	0.5	Walnut	0.3	Trout	0.0	Salt	1.4		

TL: Test line (arbitrary units, a.u.). The threshold value for considering a sample as positive was calculated as the mean TL value of the basic ingredients (except mollusks and insects) plus 3 times the SD, being 2.5 a.u.

SENSITIVITY: PROBABILITY OF DETECTION METHOD

	µg/ml	Naked eye			Strip reader			
		N	X	POD	X	TL	SD	POD
Tropomyosin	1	6	6	1.00	6	138.0	5.1	1.00
	0.5	6	6	1.00	6	134.8	2.8	1.00
	0.1	6	6	1.00	6	74.8	7.7	1.00
	0.05	40	40	1.00	40	39.0	4.4	1.00
	0.025	40	40	1.00	40	14.9	1.9	1.00
	0.0125	40	40	1.00	40	6.8	1.2	1.00
	0.00625	20	15	0.75	8	2.4	0.7	0.40
	0	6	0	0.00	0	0.5	0.4	0.00
Crustacean protein	10000	6	6	1.00	6	127.2	6.7	1.00
	1000	6	6	1.00	6	104.3	4.6	1.00
	100	6	6	1.00	6	116.9	4.7	1.00
	50	6	6	1.00	6	94.4	6.9	1.00
	10	40	40	1.00	40	27.8	2.7	1.00
	5	40	40	1.00	40	9.2	1.7	1.00
	2.5	40	36	0.90	31	3.3	1.0	0.78
	1.25	6	0	0.00	0	0.8	0.4	0.00
	0	6	0	0.00	0	0.6	0.6	0.00

TL: Test line (arbitrary units, a.u.). SD: Standard deviation. N: Number of samples analyzed. X: Number of positive samples. POD: Probability of detection (X/N).

ANALYSIS OF MODEL FOOD INCURRED WITH GROUND SHRIMP

	Frankfurt sausages	Chicken croquettes	Chicken broth
	Cooking 75 °C/40 min Pasteurization 95 °C/25 min	Cooking 100 °C/8 min	Sterilization 20 min
Shrimp protein (µg/g)	400 50 20 10 5 2.5 0	400 50 20 10 5 2.5 0	400 50 20 10 5 2.5 0
Naked eye	+ + + + - - -	+ + + + - - -	+ + + + - - -
Strip reader	144.8 24.8 12.6 7.0 2.0 0.4 0.4	159.6 37.4 7.8 4.8 1.2 0.9 0.4	152.5 64.6 16.2 7.2 2.4 0.9 0.6

CONCLUSIONS

- The developed LFIA could detect 0.0125 µg/ml of tropomyosin and 5 µg/g of crustacean protein.
- No cross-reactivity was found with a panel of 54 food ingredients, with the exception of mollusks and insects.
- The LFIA was able to detect crustaceans in model food (Frankfurt sausages, croquettes and broth) incurred with ground shrimp at levels of 10 µg/g protein.
- The use of this immunoassay could improve allergen risk management plans in the food industry, preventing the abusive use of precautionary labeling.